Detection of Chlamydiaceae in ocular swabs from Australian pre-export feedlot sheep

Short contribution

Detection of *Chlamydiaceae* in ocular swabs from Australian pre-export feedlot sheep

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Abstract

Infectious Ovine Keratoconjunctivitis (IOK) is a contagious ocular disease of sheep. A range of organisms have been observed as the aetiological agents of IOK. In this study, the presence of chlamydial pathogens (*C. pecorum, C. abortus, C. psittaci*) in conjunctival swabs was tested for. The swabs were collected from sheep with varying grades of IOK in an Australian pre-export feedlot. The sheep had been rejected from a shipment because of the eye disease. The relative contribution of chlamydial pathogens to IOK and the rejection of animals was evaluated.

In total, 149 conjunctival swabs were taken from rejected sheep (IOK Grades 1 to 6; n=126) as well as those with healthy eyes (Grade 0; n = 23). Screening for chlamydial pathogens was done using species–specific qPCR assays. Chlamydial DNA was detected in 35.6% (53/149) of conjunctival samples. *C. pecorum* was the most predominant species with an overall prevalence of 28.9% (43/149). *C. psittaci* prevalence was 6.7% (10/149). Both organisms were detected in healthy as well as IOK-affected eyes. All swabs tested negative for *C. abortus.*

The results from this study demonstrate that *Chlamydia* spp. can be readily detected in sheep presenting with IOK. The zoonotic *C. abortus* was not detected in any of the samples in this study, providing further evidence to the suggestion that this pathogen remains absent from Australia. Although the exact contribution of *Chlamydia* spp. in the IOK pathogenesis is unclear, such studies are anticipated to be of benefit to Australian domestic and live export production systems.

Keywords: Sheep; Infectious keratoconjunctivitis; *Chlamydia*; species-specific qPCR; *Chlamydia pecorum*; *Chlamydia psittaci*

Abbreviations: IOK, Infectious Ovine Keratoconjunctivitis; qPCR, quantitative PCR; HRM, high resolution melt; CI, confidence interval.

Introduction

Infectious Ovine Keratoconjunctivitis (IOK), also known as *pinkeye*, is a highly transmissible ocular disease of sheep that has a worldwide distribution. IOK is characterised by an acute conjunctivitis, blepharospasm, lacrimation, and hyperaemia, followed by varying degrees of corneal opacity and ulceration[1]. A range of organisms, including *Mycoplasma* spp. (notably *M conjunctivae*), *Moraxella ovis,* and *Chlamydia* spp. have been observed as the aetiological agents of IOK[1-4].

In Australia, besides the concerns for animal welfare, diseases such as IOK can also cause significant economic loss to the ovine feedlot and export industry associated with animal rejections from shipments[2]. A study characterising the ocular flora associated with IOK as well as the flora in healthy eyes in pre-export feedlot sheep in Western Australia, identified a range of microorganisms. *Mycoplasma* spp. and *Moraxella ovis* were the pathogens most closely associated with IOK. However, it was also observed that healthy animals shed these same pathogens, representing a possible a risk to other sheep [2]. At the time of the study, no testing was performed for *Chlamydia* spp.

Over the past decade, several studies have demonstrated that the obligate intracellular bacterial pathogen, *Chlamydia pecorum*, can cause economically significant diseases such as conjunctivitis and arthritis in Australian sheep, as recently reviewed [4]. *Chlamydia abortus*, the important cause of ovine enzootic abortion in small ruminants elsewhere in the world is seemingly absent in Australia[5]. The avian pathogen, *Chlamydia psittaci* infects birds and has occasionally been documented in mammals, including humans[6, 7] . While laboratory detection of chlamydial infections in domestic sheep production is primarily limited to *C. pecorum*, some countries importing Australian livestock for breeding purposes require *C. abortus* testing, despite no confirmatory evidence of *C. abortus* infection in Australian animals. This latter testing relies primarily on commercially available assays with low specificity and hence high cross-reactivity to other *Chlamydiae*[8] as well as other bacteria [5]. The result is that the specific prevalence and impact of these chlamydial infections on the health of live export animals is unknown.

In this study, retrospective screening for several chlamydial pathogens (*C. pecorum, C. abortus, C. psittaci*) in conjunctival swabs collected from pre-export rejected sheep with varying grades of IOK from an Australian pre-export feedlot was performed. It was hypothesised that chlamydial pathogens are commonly detected in IOK and such infections may contribute to the IOK disease process and are therefore associated with rejection of animals before export.

Materials and methods

One hundred and twenty seven conjunctival swabs were collected from sheep with naturally occurring IOK that arrived at a pre-export feedlot in Western Australia. The sheep were selected from a group of sheep that had been identified as unsuitable for shipment on arrival at the feedlot within the previous three days. This group was made up of animals with a variety of ailments, not just ocular lesions. Those with ocular lesions were identified initially and then a further selection was taken from this group to be included in the study. The conjunctival swabs were taken from sheep with varying degrees of IOK ranging from epiphora (weeping eye) (Grade 1; n = 32), conjunctivitis and scleral injection (Grade 2; n = 49), corneal oedema (Grade 3; n = 28), corneal ulceration (Grade 4; n = 9), corneal neovascularisation (Grade 5; n = 7) and to chronic eye damage (Grade 6; n =1). This swab collection was followed by heterotrophic bacterial isolation and identification. Cotton tipped swabs were placed between the globe and the conjunctiva and gently rotated for 15 seconds. The study was carried out with approval from Murdoch University's Animal Ethics Committee (AEC R2613/13).

Twenty-three sheep with healthy "normal" eyes, showing no clinical signs of IOK, were selected and sampled from Murdoch University's teaching flock. These animals were selected from the merino adult breeding ewe group. The animals selected had no history of ocular disease in the past twelve months and there had been no history of recent treatment with antibiotics. These conjunctival swabs taken from sheep with healthy, clinically unaffected eyes were used as a control in our study (Grade 0; n = 23).

The swabs (n=149) were processed by vortexing and heating to 95^{o}C , followed by DNA extraction using a QIAmp DNA kit (Qiagen, Doncaster, Australia)[9]. The use and testing of these

swabs was considered and approved for exemption by the University of The Sunshine Coast (USC) Animal Ethics Committee (AN/E/14/01).

All 149 samples were screened for chlamydia using species–specific qPCR assays. *C. pecorum*-specific qPCR targeted a 209 bp region of the *C. pecorum* CpecG_0573 gene characterised with a high-resolution melt (HRM) of 77.5 ± 0.5 ^{o}C, while *C. psittaci*-specific qPCR assay targeted a 263 bp fragment of the Cps_ORF607 gene characterised with a melt of 79 ± 0.5 ^{o}C [10]. The *C. abortus*-specific qPCR assay targeted a 190 bp of the Cab_ORF581 gene[11], characterised with a melt of 77 ± 0.5 ^{o}C. Each assay was calibrated using known standards of *C. pecorum*, *C. psittaci* and *C. abortus* target amplicons serially diluted from 10^6 to 10^0 copies/µl. The amplicons were generated by conventional PCR from purified genomic DNA of the *C. pecorum* E58 bovine isolate, *C. psittaci* CR009 parrot isolate and *C. abortus* B577 ovine isolate, respectively. Each sample was tested in duplicate, with negative (dH_20) and positive controls included in each amplification assay. Samples with <10 copies/µl (and/or >Ct 33) and below the signature melt threshold were considered negative.

To evaluate the relationship between each IOK grade and chlamydial positivity, statistical analyses was performed using the Chi-squared test for r x c contingency table with 95% confidence. To compare the species prevalence proportions, we used the two tailed 2-sample z-test with 95% confidence, as implemented in the online epidemiological calculator software EpiTools (http://epitools.ausvet.com.au/content.php?page=StatisticsHome) [12].

Results

Chlamydial DNA was detected in 35.6% (53/149) of conjunctival samples. *C. pecorum* was the most predominant species with *C. pecorum*-specific PCR screening revealing an overall prevalence of 28.9% (43/149). *C. pecorum* DNA was detected in swabs from animals with "healthy" unaffected eyes as well as all grades of ocular pathology except for Grade 5 (Table 1). Excluding the Normal grade samples, *C. pecorum* prevalence in IOK samples was 28.5% (36/126). There was no statistically significant association between *C. pecorum* positivity and IOK severity.

137 *C. psittaci* was detected in a total of 6.7% (10/149) samples from both normal as well as IOK
138 affected eyes. The relationship between *C. psittaci* positivity and different IOK grades was also
139 not statistically significant (Table 1). Detection of *C. psittaci* in animals with IOK was only 5.6%
140 (10/126), significantly lower than the overall prevalence of 28.5% for *C. pecorum* in IOK (P
141 <0.0001).
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143 All swabs tested negative for *C. abortus*.

Conclusion

The results from this study demonstrate that *Chlamydia* spp. and, in particular, *C. pecorum* can be readily detected in sheep presenting with IOK, but also in animals with no evidence of ocular disease. Consistent with recent pilot molecular studies[8] of Australian sheep, no evidence for *C. abortus* infection could be found.

The high number of *C. pecorum* positives is consistent with the reported endemicity of *C. pecorum* infections in Australian sheep [4, 8, 13]. The majority of these infections are typically reported to be asymptomatic in adult animals , while serious disease (in a form of keratoconjunctivitis, polyarthritis and/or encephalomyelitis) appears to be mainly limited to lambs and calves [8, 13]. Whilst the results of this study show that chlamydial organisms can be shed from both healthy as well as clinically affected eyes of sheep, there was no obvious statistical relationship between their presence in healthy sheep as well as the different stages of IOK severity (Table 1). The exact contribution of *C. pecorum* to pathogenesis of these cases of IOK is unclear and disease in animals is likely to be multi-factorial, including co-infections with other microorganisms, the prevaling environmental conditions and host-related factors.

The detection of *C. psittaci* was surprising as revealed a new livestock host for this pathogen in Australia, but not completely unexpected. Infections in Australian horses have been recently documented for the first time and have been linked to reproductive loss in mares [7]. Molecular typing of these cases suggested that infection spill-over from psittacine birds may be the source [7]. Globally, *C. psittaci* infections have been documented in asymptomatic sheep as well as sheep with ocular disease before [3, 14]. At present, the genetic identity of these ovine *C. psittaci* strains is mainly unknown, however limited molecular typing revealed that ovine *C. psittaci* strains are also closely related to the psittacine and other birds[7]. On the basis of PCR testing alone, this result should be treated with caution with further work required to establish whether these results are evidence of *C. psittaci* conjunctival infection or simply exposure to *C. psittaci* DNA present in the environment potentially contaminated with bird feces.

In a pilot study, it was shown that commercially available *C. abortus*-specific assays demonstrate low specificity, with high rates of seropositivity in animals with concurrent and

highly prevalent *C. pecorum* infection in the absence of any detectable *C. abortus* infection[8] . As such, the use of these assays may be contributing to unnecessary rejection of animals where *C. abortus* testing is required. The failure to detect any *C. abortus* positive samples from the current study is consistent with this recent pilot work . An obvious weakness of this approach is that conjunctival sampling is not optimal for the detection of *C. abortus*, although *C. abortus* conjunctival infections have been documented [3, 15]. Further and expanded studies of *C. abortus* prevalence in Australian livestock are required to resolve any uncertainty over *C. abortus* infection, particularly those including paired sampling of sera and mucosal sites (conjunctiva, reproductive organs, rectum) for chlamydial detection. Such studies are anticipated to be of benefit to Australian domestic and live export production systems.

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Conflict of interest and funding

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Table 1. *C. pecorum*, *C. psittaci* and *C.abortus* detection in the eye swabs taken from sheep with varying IOK grades.

IOK affected eye grade	**No. of samples**	***C. pecorum* (%)**	**Chi square* (P value, 95% CI)**	***C. psittaci* (%)**	**Chi square* (P value, 95% CI)**	***C. abortus* (%)**
0 - Normal	23	7/23 (30.4)	-	3/23 (13.0)	-	0/23 (0.0)
1 - Epiphora	32	8/32 (25.0)	0.33	0/32 (0.0)	N/A	0/32 (0.0)
2 - Conjunctivitis	49	16/49 (32.6)	0.18	2/49 (4.1)	0.19	0/49 (0.0)
3 - Oedema	28	9/28 (32.7)	0.21	4/28 (14.3)	0.06	0/28 (0.0)
4 - Ulceration	9	2/9 (22.2)	0.57	1/9 (11.1)	0.5	0/9 (0.0)
5 - Neovascularisation	7	0/7 (0.0)	N/A^	0/7 (0.0)	N/A	0/7 (0.0)
6 - Damage	1	1/1 (100.0)	0.23	0/1 (0.0)	N/A	0/1 (0.0)
Total	**149**	**43 (28.9)**		**10 (6.7)**		**0 (0.0)**

*: IOK grade compared to normal; ^N/A: not applicable.